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(54) Title: NOVEL PROCOLLAGENS

(57) Abstract

There is disclosed molecules comprising at least a first moiety having the activity of a procollagen C-propeptide and a second moiety selected from any one of the group of an alien collagen α-chain and non-collagen materials, the first moiety being attached to the second moiety. Also disclosed are collagen molecules, fibrils and fibres comprising a non-natural combination of collagen α-chains. DNA encoding same, expression hosts transformed or transfected with same, transgenic animals and methods of producing a non-natural collagen.

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Novel Procollagens

The present invention concerns novel molecules, in particular novel procollagen molecules, together with collagen molecules, fibrils and fibres comprising a non-natural combination of collagen α-chains, DNA encoding same, expression hosts transformed or transfected with same, transgenic animals and methods of producing a non-natural collagen.

Collagen (also known as processed procollagen molecule and triple helical processed procollagen monomeric molecule) (for general reviews see Kadler, K., 1995, Protein Profile, "Extracellular Matrix 1: fibril-forming collagens", 2: 491-619; Ayad, S. *et al.*, 1994, The Extracellular Matrix Facts Book, Academic Press, London, ISBN 0-12-068910-3 and references therein) is a major structural protein in animals where it occurs in the extracellular matrix (ECM) of connective tissues, mostly in the form of fibrils (also known as polymeric collagen). The collagen fibrils (polymeric collagen) are the major source of mechanical strength of connective tissues, providing a substratum for cell attachment and a scaffold for dynamic molecular interactions. The family of collagens comprises complex multidomain proteins comprising three collagen α-chains wound into a triple helix. At least twenty genetically-distinct collagen types have been described to date and they can be classified into subgroups on the basis of gene homology and function of the encoded protein. Fibril-forming collagens (types I, II, III, V and XI; see Table 1) are synthesized as soluble procollagens (also known as proα chains, procollagen α-chains and monomer chains) and comprises a C-propeptide, a Gly-X-Y repeat containing region (which in the case of monomer chains of fibril-forming collagens comprise an uninterrupted collagen α-chain) and an N-propeptide. The proα chains trimerise to form unprocessed procollagen molecules (also known as monomeric procollagen molecules and trimerised proα chains), assembling into fibrillar structures upon enzymic cleavage of their N- and C-terminal propeptide domains (the N- and C-propeptides) (see Figure 1).

Although the genes encoding the proα chains are remarkably similar, relatively little is known about the processes which control the folding and trimerization of the proα chains (Dion, A.S. and Myers, J.C., 1987, J. Molec. Biol., 193: 127-143), and only a restricted range of collagens is formed. For example, skin fibroblasts synthesise co-incidentally the six highly homologous proα chains (proα1(I), proα1(III), proα1(V), proα2(I), proα2(V) and proα3(V)). Despite the great number of possible combinations of the six proα chains, only specific combinations of collagen chains occur - these are those resulting in types I, III and V collagen. Type I collagen exists as a heterotrimer and assembles with the stoichiometry of two proα1(I) chains and one proα2(I) chain ($[\text{pro}\alpha 1(\text{I})]_2$ proα2(I)). Homotrimers of proα2(I) have not been detected and hence the inclusion of this chain in a trimer is dependent upon its association with proα1(I) chains. Type III collagens comprise a homotrimer ($[\text{pro}\alpha 1(\text{III})]_3$), and the constituent chains do not assemble with either of the Type I collagen proα chains. Type V collagen displays heterogeneity with regard to chain composition, forming both homo- ($[\text{pro}\alpha 3(\text{V})]_3$) and hetero-trimers ($[\text{pro}\alpha 1(\text{V})]_2$ proα2(V) and [proα1(V) proα2(V) proα3(V)]).

The C-propeptide is known to be implicated in the assembly of the monomer chains into trimerised proα chains (unprocessed procollagen) prior to cleavage of the N- and C-propeptides and formation of collagen in fibril-forming proα chains. The assembly of the three monomer chains into trimerised proα chains is initiated by association of the C-propeptides. This association can be divided into two stages: an initial recognition event between the proα chains which determines chain selection and then a registration event which leads to correct alignment and folding of the triple helix. Comparison (Figure 2) of the amino acid sequences of the C-propeptides of proα1(I), proα2(I) and proα1(III) proα chains, which assemble to form collagen types I and III, demonstrates the striking level of sequence similarity between these proα chains yet, despite the homology, they invariably assemble and fold in a collagen type-specific manner.

It has now been found that the C-propeptides, and more particularly certain sequences within them, are not only necessary but are also sufficient to determine the type-specific assembly of the moieties to which they are attached. because of the presence of these certain sequences, the C-propeptides are capable of autonomously directing the assembly of the attached moieties, which in particular may be an alien collagen α-chain. The present inventors have isolated and characterised a region of the C-propeptide which defines the chain selection event but which does not affect the subsequent folding. This has allowed the synthesis of novel proα chains which have formed novel trimerised proα chains and collagen. Now that the chain selection interactions between the proα chains can be controlled, a vast range of novel trimeric molecules, in particular collagens, may be synthesised at will using existing and novel proα chains and C-propeptides. These new molecules may possess selected biological and physical properties and have a wide range of uses. For example, novel collagens may be used in industries which use collagen either as a product or as part of a process. Such collagens and uses may include for example: novel gelatins for use in food, food processing and photography; novel finings for clearing yeast during the brewing process; novel gelatins for the food packaging industry; novel polymers for the manufacture of textiles; novel glues for use in construction, building and manufacturing; novel coatings for tablets; novel glues for use with the human or animal body; novel collagens for use as body implants; novel collagens and procollagens as adjuvants; novel collagens and procollagens as molecular carriers for drugs and pharmaceuticals; and as modulators of collagen fibril formation for use in. for example, wound healing and fibrosis.

According to the present invention there is provided a molecule comprising at least a first moiety having the activity of a procollagen C-propeptide and a second moiety selected from any one of the group of an alien collagen α-chain and non-collagen materials, the first moiety being attached to the second moiety.

The molecule may be able to bind to other similar molecules. It may trimerise with other similar molecules.

The first moiety will generally be attached to the C-terminal end of the second moiety, although intervening amino acid residues may be present.

The first moiety may comprise a proα chain C-propeptide or a partially modified form thereof or an analogue thereof, and when forming the C-terminal region of a proα chain, may allow the molecule to bind to other similar molecules. The C-propeptide region of a proα chain may be the C-terminal fragment resulting from C-proteinase cleavage of a proα chain. The C-proteinase may cleave between the residues G and D or A and D or an analogue thereof in the sequence FAPYYGD (residues 376-382 of SEQ ID NO: 2), YYRAD (residues 1-5 of SEQ ID NO: 14) or FYRAD (residues 284-288 of SEQ ID NO: 1) (Figure 2) or an analogue thereof.

Modifications to molecules may include the addition, deletion or substitution of residues. Substitutions may be conservative substitutions. Modified molecules may have substantially the same properties and characteristics as the molecules form which they are derived. Modified molecules may be homologues of the molecules from which they are derived. They may for example have at least 40% homology, for example 50%, 60%, 70%, 80%, 90% or 95% homology.

The present inventors have isolated and identified (see "Experimental" section) a site - the recognition site - in the procollagen C-propeptide which contains a sequence which is necessary and sufficient to determine the type-specific assembly of the moieties to which it is attached. The recognition site is defined as being the part of the C-propeptide containing the sequence (the recognition sequence) which, in an alignment plot of the C-propeptide against other C-propeptides, corresponds to the sequence in the region between the junction points B and G (Figure 2). Alignment plots may be done using the

PILE-UP program on SEQNET at the Daresbury Laboratories, UK. An existing proα chain which has been substituted at the recognition sequence and as a result has different properties or characteristics is considered to be a molecule comprising a proα chain C-propeptide and an alien collagen α-chain since the C-propeptide is novel, all collagen α-chains therefore being alien to it.

As can be seen from Figure 2, the recognition sequences contain a region of homologous amino acids. Substitution to the conserved residues (see "Experimental" section below) has not disrupted chain selection nor has it prevented the formation of a correctly aligned helix, and so it appears that the conserved residues are not involved in chain selection. Hence the recognition sequence, although comprising a continuous sequence of about 23 amino acids, may be considered to have the chain selection properties contained within a discontinuous variable sequence. For example in the recognition sequence of alpha 1(III) (SEQ ID NO: 6) the discontinuous variable sequence may be considered to comprise residues 1-12 and 21-23.

The C-propeptide and/or the recognition sequence may be that of a fibrillar proα chain. More generally, the C-propeptide may be an existing C-propeptide, for example a C-propeptide found in nature, or it may be a partially modified form of or an analogue (i.e. possessing substantially the same properties and characteristics but having a different sequence) of an existing proα chain C-propeptide, or it may comprise a novel C-propeptide (i.e. a C-propeptide having significantly different properties or characteristics to other C-propeptides) and may for example have different binding kinetics or α-chain selection properties.

The existing C-propeptide may be selected from any one of the group of the proα1(I), proα2(I), pro1(II), proα1(III), proα1(V), proα2(V), proα3(V), proα1(XI), proα-2(XI), and proα3(XI) proα chain C-propeptides or a partially modified form thereof or an analogue thereof. Partially modified forms of procollagen C-propeptides include the

recognition sequences of C-propeptides, for example those identified in Figure 3 of the accompanying drawings in relation to the C-propeptides from which they are derived. In some embodiments of the invention, such modified forms may be the only, or substantially the only, elements derived from a C-propeptide, in other words, no other C-propeptide-derived sequences need be present. However, this will not always be the case, as the invention also encompasses the presence of other parts of the C-propeptide including, of course, the balance of it.

The C-propeptide may comprise an existing C-propeptide or a partially modified form thereof or an analogue thereof substituted at the recognition site. The C-propeptide may be substituted at the recognition site with the recognition sequence of an existing C-propeptide, for example that of a different C-propeptide. It may for example be substituted at the recognition site with the recognition sequence of the C-propeptide of any one of the group of proα1(III), proα1(I), proα2(I), proα1(II), proα1(V), proα2(V), proα1(XI), proα2(XI) and proα3(XI) proα chains. It may be substituted at the recognition site with a recognition sequence having the sequence of any one of the group of SEQ ID NOS: 6-13. The recognition sequence of a C-propeptide which has been modified for example by addition, deletion or substitution of amino acid residues yet which has substantially the same properties and/or characteristics is considered to be essentially that of an existing C-propeptide. The recognition sequence may generally be at least 40% homologous, or even at least 50, 60, 70, 80, 90 or 95% homologous to the sequence from which it was derived.

Such a substitution at the recognition site may significantly affect the properties or characteristics of the C-propeptide

Alternatively, the recognition sequence may be novel. Such a novel recognition sequence may for example give the first moiety novel binding kinetics or specificity for a novel first moiety or a novel set of first moieties.

The second moiety is a molecular component which may be anything bound to the first moiety. This may include, for example, alien collagen α-chain molecules, or other proteins or fragments of proteins, such as antibodies or antigen binding fragments thereof, or combinations thereof. Proteins constituting or contributing to the second moiety may be glycosylated or otherwise post-translationally modified. By "alien collagen α-chain" is meant a collagen α-chain which does not form part of a proα chain with the C-propeptide in nature; collagen α-chains comprise a triple helical forming domain, and an N-propeptide may also be present. Other collagen α-chains from the same species, as well as those from different species, may be used. Included as collagen α-chains which do not form part of a proα chain with the C-propeptide in nature are partially modified forms and analogues of existing collagen α-chains which form part of a proα chain with the C-propeptide in nature and which do not significantly affect the relevant properties or characteristics of the procollagen molecule, such as binding specificity. Partially modified forms and analogues of collagen α-chains may, for example, have additions, deletions or substitutions which do not significantly affect the relevant properties or characteristics of the C-propeptide or collagen α-chain.

By means of the invention, therefore, novel collagens may be produced. Such novel collagens have combinations of α-chains which are not seen in nature because of the assembly-directing effect of the natural C-propeptides. The invention allows the protein engineer to construct novel collagens having a non-natural combination of α-chains. The invention therefore extends to a procollagen molecule comprising a non-natural combination of α-chains. Non-natural pro-collagen homotrimers and heterotrimers, including all the possible trimers not mentioned in Table 1, are within the scope of the invention.

The second moiety may comprise at least a collagen α-chain. A collagen α-chain may be selected from any one of the group of proα1(I) chain, proα2(I) chain,

proα1(II) chain, proα1(III) chain, proα1(V) chain, proα2(V) chain, proα3(V) chain, proα1(XI) chain, proα2(XI) chain, and proα3(XI) chain collagen α-chains.

The second moiety may also comprise a proα chain N-propeptide. An N-propeptide may be selected from any one of the group of the proα1(I), proα2(I), proα1(II), proα1(III), proα1(V), proα2(V), proα3(V), proα1(XI), proα2(XI), and proα3(XI) proα chain N-propeptides.

The second moiety may comprise a collagen α-chain and N-propeptide which are naturally associated (for example those of the proα2(I) chain), or it may comprise a non-natural combination of collagen α-chain and N-propeptide. Depending upon the host organism in which it may be desired to express molecules of the invention, the N-terminal propeptide may be replaced or adapted to facilitate secretion or other handling or processing in the expression system.

The molecule may comprises a first moiety having the activity of the proα1(III) C-propeptide attached to a second moiety comprising the collagen α-chain and N-propeptide of the proα2(I) chain. The molecule may have the sequence of SEQ ID NO: 4.

In the natural formation of a collagen molecule *in vivo*, the N- and C-propeptides are cleaved off the procollagen molecule to yield a collagen molecule during the formation of polymeric collagen. Consequently, the invention includes within its scope a collagen molecule comprising a non-natural combination of α-chains. Non-natural collagen homotrimers and heterotrimers, including all the possible collagen trimers not mentioned in Table 1, are within the scope of the invention. (If for any reason it is desired to have a non-natural collagen molecule with a C-propeptide but not an N-propeptide, or *vice versa*, the enzymes responsible for processing in the chosen expression system may be

manipulated or selected accordingly or the sequence of the molecule modified to make it susceptible to enzymatic processing as appropriate.)

Collagen molecules naturally self-assemble into collagen fibrils, which in turn aggregate to form a collagen fibre. Collagen fibrils and collagen fibres comprising collagen molecules as described above are therefore also contemplated by the invention.

Molecules of the first aspect of the invention may be prepared by any convenient method, including peptide ligation and complete synthesis. It is preferred however, that the molecules be prepared by expression from a recombinant DNA system. For this purpose, and according to a second aspect of the invention, there is provided a DNA molecule, which may be in recombinant or isolated form, encoding a molecule as described above (particularly a non-natural procollagen α-chain).

Recombinant DNA in accordance with the invention may be in the form of a vector. The vector may for example be a plasmid, cosmid or phage. Vectors will frequently include one or more selectable markers to enable selection of cells transfected (or transformed: the terms are used interchangeably in this specification) with them and, preferably, to enable selection of cells harbouring vectors incorporating heterologous DNA. Appropriate start and stop signals may be present. The vector may be an expression vector having regulatory sequences to drive expression. Vectors not including regulatory sequences are useful as cloning vectors; and, of course, expression vectors may also be useful as cloning vectors.

Cloning vectors can be introduced into *E. coli* or another suitable host which facilitate their manipulation. According to another aspect of the invention, there is therefore provided a host cell transfected or transformed with DNA as described above. Such host cells may be prokaryotic or eukaryotic. Eukaryotic hosts may include yeasts, insect and mammalian cell lines. Expression hosts may be stably transformed. Unstable and cell-free

expression systems may be used in appropriate circumstances, but it is unlikely that they will be favoured, at the present state of technology, for bulk production.

DNA of the invention may also be in the form of a transgene construct designed for expression in a transgenic plant or, preferably, animal. In principle, the invention is applicable to all animals, including birds such as domestic fowl, amphibian species and fish species. The protein may be harvested from body fluids or other body products (such as eggs, where appropriate). In practice, it will be to (non-human) mammals, particularly placental mammals, that the greatest commercially useful applicability is presently envisaged, as expression in the mammary gland, with subsequent optional recovery of the expression product from the milk, is a proven and preferred technology. It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels and pigs that the invention is likely to be most useful. The generation and usefulness of such mammalian transgenic mammary expression systems is both generally, and in certain instances specifically, disclosed in WO-A-8800239 and WO-9005188. The β-lactoglobulin promoter is especially preferred for use in transgenic mammary expression systems. WO-A-9416570 purports to disclose the production of human recombinant collagen in the milk of transgenic animals but contains no experimental details of such production having taken place.

Expression hosts, particularly transgenic animals, may contain other exogenous DNA to facilitate the expression, assembly, secretion and other aspects of the biosynthesis of molecules of the invention. For example, expression hosts may co-express prolyl 4-hydroxylase, which is a post-translational enzyme important in the natural biosynthesis of procollagens, as disclosed in WO-9307889.

DNA, particularly cDNA, encoding natural procollagen chains is known and available in the art. For example, WO-A-9307889, WO-A-9416570 and the references cited in both of them give details. Such DNA forms a convenient starting point for DNA of the

present invention, which may be prepared by recombinant techniques from it. While in general terms DNA encoding a C-propeptide (or a minimal essential region from it) may simply be ligated to DNA encoding an alien collagen triple helical domain (usually attached to DNA encoding the corresponding N-propeptide), in practice it is useful to use PCR-based techniques to effect the precise ligation. For example, PCR products flanking the junction region between the C-propeptide and the triple helical domain may be prepared and combined; an overlap extension reaction can then be carried out to yield a PCR product which is a hybrid between DNA encoding the C-propeptide of one procollagen chain and DNA encoding the triple helical domain (and the N-propeptide, usually) of another procollagen chain.

The invention is in principle capable of accommodating the use of synthetic DNA sequences, cDNAs, full genomic sequences and "minigenes", which is to say partial genomic sequences containing some, but not all, of the introns present in the full length gene. Because of the large number of introns present in collagen genes in general, though, experimental practicalities will usually favour the use of cDNAs or, in some circumstances, minigenes.

DNA in accordance with the invention can in principle be prepared by any convenient method involving coupling together successive nucleotides, and/or ligating oligo- and/or poly-nucleotides, including *in vitro* processes, but recombinant DNA technology forms the method of choice.

Molecules of the invention may be useful in a method of treatment or diagnosis of the human or animal body. The invention therefore extends to molecules as described above for use in medicine.

The molecule may be for use as an adhesive or implant. It may be for use in promoting the healing of wounds or fibrotic disorders with reduced scarring. It may be for

use in promoting the healing of chronic wounds. By "wounds or fibrotic disorders" is meant any condition which may result in the formation of scar tissue. In particular, this includes the healing of skin wounds, the repair of tendon damage, the healing of crush injuries, the healing of central nervous system (CNS) injuries, conditions which result in the formation of scar tissue in the CNS, scar tissue formation resulting from strokes, and tissue adhesion, for example, as a result of injury or surgery (this may apply to e.g. tendon healing and abdominal strictures and adhesions). Examples of fibrotic disorders include pulmonary fibrosis, glomerulonephritis, cirrhosis of the liver, and proliferative vitreoretinopathy.

For example in the inhibition of fibrosis, a novel collagen molecule or proα chain may be applied to a site of wounding or fibrosis, the novel collagen (or proα chain) inhibiting collagen fibril formation and thus fibrosis. The novel collagen or proα chain may for example have a shortened α-chain.

DNA of the invention may be useful, in appropriate constructs, in a method of gene therapy. It may be for use in the treatment of Osteogenesis Imperfecta (OI), Ehlers-Danlos Syndrome (EDS), Stickler Syndrome, Spondyloepiphyseal dysplasia, Hypochondrogenesis or Aortic Aneurisms. Mutations within collagen genes are the cause of most forms of OI, some forms of EDS and of some chondrodysplasias. In most cases the devastating effects of the disease are due to substitutions of glycine within the triple helical domain - the Gly-X-Y repeat containing region - for amino acids with bulkier side chains. This results in triple helix folding being prevented or delayed with the consequence that there is a drastic reduction in the secretion of trimerised proα chains. The malfolded proteins may be retained within the cell, probably within the ER (endoplasmic reticulum), where they are degraded. As the folding of the C-propeptide is not affected by these mutations within the triple helical domain, C-propeptides from wild-type as well as mutant chains associate and may be retained within the cell. The retention and degradation of wild-type chains due to their interaction with mutant chains amplifies the effect of the mutation and has been termed "procollagen suicide". The massive loss of protein due to this

phenomenon may explain the dominant lethal effects of such mutations. By engineering proα chains having altered chain selectivity, proα chains maybe produced which do not associate with the mutant chains, and will therefore fold and be secreted normally. Such engineered proα chains may contain the wild-type collagen α-chain, thereby making up for the deficit caused by the mutant collagen α-chain. Expressed protein may in some circumstances also be useful in the treatment of diseases and conditions which could be addressed at a more fundamental level by gene therapy.

The invention may also be useful in photography, brewing, foodstuffs, textiles or adhesives.

Also provided according to the present invention is a method of treatment or diagnosis of the human or animal body comprising the use of a molecule according to the present invention.

The invention will be further apparent from the examples, which comprise the following Figures and description of experiments, of which:

Figure 1 shows the initial stages in the intra-cellular folding, assembly and modification of procollagen. As can be seen, co-translational translocation and signal peptide cleavage occurs at stage number 1. Intra-molecular disulphide bond formation then takes place as well as N-linked glycosylation, proline isomerisation and proline hydroxylation at stage 2. There then follows at stage 3 type-specific assembly of the proα chains by trimerisation and inter-molecular disulphide bond formation. Finally, at stage 4, triple helix formation proceeds in a carboxy-to-amino-direction to give trimerised proα chains;

Figure 2 shows an alignment plot made using the PILE-UP program on SEQNET at the Daresbury Laboratories, UK using default settings, of the C-propeptides

of proα chains of type I and type III procollagen. Alpha 1(I) is SEQ ID NO: 14; alpha 2(I) is residues number 284-534 of SEQ ID NO: 1; and alpha 1(III) is residues number 379-626 of SEQ ID NO: 2. C-proteinase cleavage sites (marked CP) are between A and D (alpha 1(I)), A and D (alpha 2(I)) and G and D (alpha 1(III)). Junction points A, F, B, C and G are as shown. Numbers indicate conserved cysteine residues. # indicates identical amino acids and ~ indicates amino acids with conserved side chains;

Figure 3 shows recognition sequences for proα1(I), proα2(I), proα1(II), proα1(III), proα1(V), proα2(V), proα1(XI) and proα2(XI) proα chains having SEQ ID NOs: 7, 8, 9, 6, 10, 11, 12, and 13 respectively and which were identified by alignment plots of C-propeptides against other C-propeptides (specifically, those of Figure 2) as corresponding to the sequences in the regions between junction points B and G of Figure 2;

Figure 4 shows an SDS-PAGE gel of translated procollagen constructs. Lanes are as follows: 1 - molecular weight markers; 2 and 6 - proα1(III)Δ1; 3 and 7 - proα2(I)Δ1; 4 and 8 - proα2(I):(III)CP; 5 and 9 - proα1(III):(I)CP;

Figure 5 shows an SDS-PAGE gel of translated procollagen constructs. Lanes are as follows: 1 - molecular weight markers; 2 and 7 - A-join; 3 and 8 - F-join; 4 and 9 - B-join; 5 and 10 - C-join; 6 and 11 - recip-C-join;

Figure 6 shows translated procollagens in the presence (Lanes 3, 5 and 7) and absence (Lanes 2, 4 and 6) of α,α'-dipyridyl. Lanes are as follows: 2 and 3 - proα2(I):(III)CP; 4 and 5 - BGR; 6 and 7 - proα1(III):(I)CP;

Figure 7 shows an SDS-PAGE gel of translated procollagen constructs under reducing (lanes 1-3) and non-reducing (lanes 4-6) conditions. Lanes are as follows: 1 and 4 - BGR^{S-C}; 2 and 5 - BGR; 3 and 6 - BGR^{L-M}; and

Figure 8 shows an SDS-PAGE gel of translated procollagen constructs. Lanes are as follows: 1 - molecular weight markers; 2 - BGR^{S-C}; 3 - BGR; 4 - BGR^{L-M}.

EXPERIMENTAL

A cDNA clone coding for a truncated proα2(I) chain (designated proα2(I)Δ1; SEQ ID NO: 1; nucleic acid coding sequence - SEQ ID NO: 19) was constructed from two partial cDNA clones, pHf1131 and pHp2010 (Kuivaniemi, H. *et al.*, 1988, Biochem. J., 252: 633-640) which were sub-cloned into the EcoRI site of pBluescript SK$^+$. A 3.4 kb fragment PstI fragment containing the vector and the 5'-terminal 0.5 kb of the gene was isolated from pHp2010 and ligated to a 1.4 kb PstI fragment derived from pHf1131 encoding the 3' terminus. The resultant recombinant, proα2(I)Δ1, has a 2.2 kb deletion in the coding sequence (Lees and Bulleid, 1994, J. Biol. Chem., 269: 24354-24360).

This construct was analysed using a semi-permeabilised (SP) HT1080 cell system as described by Wilson *et al.* (1995, Biochem J. 307: 679-687). Semi-permeabilised cells were prepared from HT1080 cells. Confluent HT1080 cells from a 75 cm^2 flask were rinsed once with PBS (phosphate buffered saline), then incubated with 2 ml of PBS containing 2.5 mg/ml trypsin for 3 minutes at room temperature. The flask was transferred to ice where 8 ml of ice-cold KHM (110 mM KOAc, 20 mM Hepes, pH 7.2, 2 mM MgOAc) was added containing 100 µg/ml soyabean trypsin inhibitor and the cells released from the plate. Cells were pelleted at 12,000 rpm for 3 minutes and resuspended in 6 ml of KHM containing 40 µg/ml digitonin (diluted from a 40 mg/ml stock in DMSO (dimethyl sulfoxide)) and incubated on ice for 5 minutes. To terminate permeabilisation 8 ml of KHM was added and cells were pelleted and resuspended in 50 mM Hepes, pH 7.2, 90 mM KOAc. After 10 minutes the cells were pelleted and resuspended in 100 µl of KHM (approximately 2 x 10^6 cells). Endogenous mRNA was removed by adding $CaCl_2$ to 1 mM and Staphylococcal nuclease to 10 µg/ml and incubating at room temperature for 12 minutes. The reaction was terminated by the addition of EGTA to 4 mM, and pelleting the cells. Semi-permeabilised cells were resuspended in 100 µl of KHM. RNA was translated using a rabbit reticulocyte lysate (FlexiLysate, Promega, Southampton, U.K.) for 1 hour at 30 °C. The translation reaction (25 µl) contained 16 µl reticulocyte lysate, 1 µl 1 mM amino

acids (minus methionine), 15 μCi L-[^{35}S] methionine, 1 μl transcribed RNA and semi-permeabilised cells (approx. 1 x 10^5). After translation, N-ethylmaleimide was added to a final concentration of 20 mM. The formation of disulphide bonds was verified by comparative gel electrophoresis on 7.5% polyacrylamide gel of translation products run under reducing and non-reducing conditions.

When analysed using this cell-free system the translation product from proα2(I)Δ1 mRNA did not self-associate to form homotrimers indicating that it does not contain the information necessary for the initial recognition event (Figure 4, lanes 3 and 7).

A cDNA clone coding for a truncated proα1(III) chain (designated proα1(III)Δ1; SEQ ID NO: 2; nucleic acid coding sequence - SEQ ID NO: 20) was constructed from a full-length type III procollagen cDNA which was constructed from two partial cDNAs, pS413 and pS31 (Ala-Kokko, *et al.*, 1989, Biochem. J., 260: 509-516). Each cDNA was subcloned into the EcoRI site of pBluescript SK$^-$. A 4.7 kb Sal I (restriction enzyme) fragment containing the vector and the 5' terminal 1.8 kb was isolated from pS413 and ligated to a 3.6 kb Sal I fragment derived from pS31 to produce proα1(III). An internal 2.5 kb XhoI fragment was excised from proα1(III) and the parental plasmid re-ligated to create proα1(III)Δ1 (Lees and Bulleid, 1994, J. Biol. Chem., 269: 24354-24360).

The translation product from proα1(III)Δ1 mRNA was able to assemble to form a homotrimer as judged by its ability to form inter-chain disulphide bonded dimers and trimers when translated in a semi-permeabilised cell-free translation system. This demonstrated that it contained the information required for self-assembly (Figure 4, lanes 2 and 6).

Hybrid cDNA clones were prepared which contained sequences derived from proα1(III)Δ1 and proα2(I)Δ1. The C-proteinase cleavage site of proα1(III)Δ1 was, for these experiments, taken to be between Ala 377 and Pro 378 of SEQ ID NO: 2, instead of

between Gly 381 and Asp 382 of SEQ ID NO: 2 as shown in Figure 2. In the first of these constructs the coding sequence for the C-propeptide from the proα1(III)Δ1 chain was replaced by that for the C-propeptide from the proα2(I) chain, the resulting chimera being designated proα1(III):(I)CP (SEQ ID NO: 3). This construct failed to self-associate when translated in the cell-free system (Figure 4, lanes 5 and 9). A reciprocal construct was made where the C-propeptide from the proα2(I)Δ1 was replaced with the C-propeptide from the proα1(III) chain with the resulting chimera designated proα2(I):(III)CP (SEQ ID NO: 4).

This construct was able to self associate to form dimers and homotrimers (Figure 4, lanes 4 and 8), demonstrating directly for the first time that all the information required for selective association resides within the C-propeptide. The construct proα1(III):(I)CP was prepared as described below. Other constructs were produced using the same approach and published sequences.

The hybrid cDNA clones were prepared using a PCR-based approach. For the construction of proα1(III):(I)CP, a PCR product was prepared from proα1(III)Δ1 with primers, one of which (SEQ ID NO: 15; JL-35) hybridised within the triple helical domain whilst the other (SEQ ID NO: 16; JL-32) hybridised with 21 nucleotides upstream from the junction point at the C-propeptide. This primer also contained an overlap of 21 nucleotides which were complimentary to the first 21 nucleotides of the C-propeptide of proα2(I)Δ1. This gave a 0.25 Kb PCR product.

A second PCR product was prepared from proα2(I)Δ1 with primers, one of which (SEQ ID NO: 17; JL-31Kpn) hybridised downstream from the stop codon for translation within the 3`-non-translated region. This primer also contained a KpnI site. The other primer (SEQ ID NO: 18; JL-36) hybridised with the first 18 nucleotides of the C-propeptide of proα2(I)Δ1. This gave a 0.85 Kb PCR product.

The two PCR products were combined and a third PCR (an overlap extension) was carried out with primers JL-35 (SEQ ID NO: 15) and JL-31Kpn (SEQ ID NO: 17) to yield a 1.1 Kb product. This was cut with XhoI and KpnI and subcloned into XhoI and KpnI cut proα1(III)Δ1 to yield proα1(III):(I)CP.

A variety of hybrid constructs were then prepared in which parts of the proα2(I)Δ1 C-propeptide sequence was replaced with the corresponding region from the proα1(III) C-propeptide. The various regions are outlined in Figure 2 with the junction points designated as A, F, B, C, and G. So for example the A-join molecule contains all of the proα2(I)Δ1 sequence up to but not including the A site (i.e. . . . DY) with all of the sequence carboxy-to this site (i.e. EI . . .) being derived from the corresponding region from the C-propeptide of the proα1(III) chain. Proα1(III) and proα2(I) C-propeptides differ in their complement of cysteine residues (and hence in their ability to form disulphide bonds), with proα2(I) lacking the Cys 2 residue (Figure 2), instead having a serine residue. In order to ease analysis under non-reducing conditions (see below) the F, B and C constructs contained a serine to cysteine mutation at the Cys 2 site of the proα2(I) chain. To ensure that this mutation played no role in chain selection, a similarly mutated construct (proα2(I):(III) BGR^{S-C} - also referred to as BGR^{S-C}; see below) was back-mutated. The back-mutated construct (i.e. proα2(I):(III) BGR - also referred to as BGR) had the same chain selectivity as its parent molecule (proα2(I):(III) BGR^{S-C}) (see below).

The A-join, F-join and B-join chimeras all assembled to form homotrimers when translated in the cell-free system (Figure 5, lanes 7, 8 and 9). However, the C-join molecule did not assemble (Figure 5, lane 10) suggesting that the recognition site for assembly was contained within the sequence carboxy-terminal to the B-site and amino-terminal to the C-site. The possibility that the lack of assembly of the C-join molecule was due to this site being within the recognition sequence for assembly could not be ruled out. Evidence that the recognition site had been disrupted was obtained when the reciprocal construct was made. This construct contained the proα1(III)Δ1 chain up to the C-site with

the rest of the C-propeptide being derived from the proα2(I) C-propeptide. No assembly occurred from this construct (recip-C-join) illustrating that the recognition site had been disrupted (Figure 5, lane 11).

The next construct made contained all of the proα2(I)Δ1 sequence apart from a short stretch of 23 amino acids between the B-site and the G-site (SEQ ID NO: 6) which were replaced with the corresponding region from the C-propeptide of proα1(III). This construct (designated BGR; SEQ ID NO: 5) was altered by site directed mutagenesis of cysteine for serine at the Cys 2 site of the proα2(I) part of the molecule (i.e. cysteine was substituted for the serine 335 residue of SEQ ID NO: 5). The resultant molecule (designated proα2(I):(III) $BGR^{S\text{-}C}$ was shown to assemble to form inter-chain disulphide bonded homotrimers when translated in the cell-free system (Figure 7, lane 4), demonstrating that this short stretch of 23 amino acids contains all the information to drive homotrimer formation.

To verify that the serine to cysteine mutation did not affect chain selection, a back mutation was made (i.e. to give proα2(I):(III) BGR) and homotrimer formation analysed. As expected, no inter-chain disulphide bonded trimers were detected (Figure 7, lane 5) as this molecule does not contain the cysteine residue required for inter-chain disulphide bond formation.

To determine whether a stable triple helix was formed after translation of the chimeric procollagens, a simple protease protection assay was carried out. This involved treating the translation products with a combination of proteolytic enzymes (trypsin, chymotrypsin and pepsin). Isolated SP-cells following translation were resuspended in 0.5 M acetic acid in 1% (v/v) Triton X-100 and incubated with pepsin (100 mg/ml) for 2 hours at 20 °C. Digestions were stopped by neutralisation with 1 M Tris base and proteins precipitated with ethanol at a final concentration of 27% (v/v). Precipitated protein from pepsin digests were resuspended in 50 mM Tris-HCl, pH 7.4 containing 0.15 M NaCl, 10

mM EDTA (ethylenediaminetetra-acetic acid) and 1% Triton X-100. Chymotrypsin and trypsin were added to a final concentration of 250 µg/ml and 100 µg/ml respectively and samples incubated at room temperature for 2 minutes. The digestion was stopped by the addition of soyabean trypsin inhibitor to a final concentration of 500 µg/ml and 5 volumes of boiling SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) sample buffer and boiling the samples for 3 minutes. The results are shown in Figures 6 and 8. The formation of a stable triple helix is characterised by the appearance of a protease resistant fragment (corresponding to the triple helical domain) after digestion. Only the products of translation of the proα2(I):(III)CP (Figure 6, lane 2) and the BGR constructs (Figure 6, lane 4; Figure 8, lanes 2 and 3) generated a protease resistant fragment which were only formed when α,α'-dipyridyl (an inhibitor of prolyl 4-hydroxylase) was not present during the translation (Figure 6). As proline hydroxylation is necessary for formation of a thermally stable triple helix, this demonstrates that a correctly folded triple helix was formed with these constructs.

This also demonstrates that although BGR was not able to form trimers stabilised by inter-chain disulphide bonds, it was able to trimerise to form a correctly aligned triple helix.

Analysis of the B-G motif from the proα1(III) and proα2(I) chains (Figure 3) shows that of the residues in the recognition sequences (Figure 3; SEQ ID NOs: 6 and 8 respectively), residues 13-20 are identical with the exception of residue 17 - Leu (L) in proα1(III) and Met (M) in proα2(I). In order to determine the role played by these residues in the chain selection process, site directed mutagenesis was used to substitute Met for Leu in the proα1(III) recognition sequence in the proα2(I):(III) BGR^{S-C} construct (designated proα2(I):(III) BGR^{L-M} - also referred to as BGR^{L-M}), i.e. residue Leu 425 of SEQ ID NO: 5 was substituted for Met, and residue Ser 335 was substituted for Cys.

Chain assembly of proα2(I):(III) BGR^{L-M} was performed as described above and the electrophoretic mobility of the chains analysed. Under non-reducing conditions this construct formed inter-chain disulphide bonds (Figure 7, lane 6), and formed protease-resistant triple helical domains (Figure 8, lane 4). The substitution of Leu for Met did not, therefore, disrupt the process of chain selection nor did it prevent the formation of a correctly aligned helix.

Table 1

Type	Chains	Molecules	Distribution
I	α1(I) α2(I)	major $[\alpha 1(I)]_2\alpha 2(I)$ minor $[\alpha 1(I)]_3$	widespread, skin, bone, tendon, ligament, cornea.
II	α1(II)	homotrimers $[\alpha 1(II)]_3$	cartilage, notochord, invertebrate disc, ear, developing bone, eye, cornea
III	α1(III)	homotrimers $[\alpha 1(III)_3]$	widespread, particularly found with type I collagen
V	α1(V) α2(V) α3(V)	heterotrimers	widespread, particularly found in cornea with type I collagen
XI	α1(XI) α2(XI) α3(XI) α3(XI)=α1(II)	heterotrimers	cartilage, cornea and vitreous

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: The Victoria University of Manchester

(B) STREET: Oxford Road

(C) CITY: Manchester

(E) COUNTRY: GB

(F) POSTAL CODE (ZIP): M13 9PL

(ii) TITLE OF INVENTION: Novel Procollagens

(iii) NUMBER OF SEQUENCES: 20

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: GB 9517773.9

(B) FILING DATE: 31-AUG-1995

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: GB 9606152.8

(B) FILING DATE: 23-MAR-1996

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: GB 9612476.3

(B) FILING DATE: 14-JUN-1996

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 535 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Met Leu Ser Phe Val Asp Thr Arg Thr Leu Leu Leu Leu Ala Val Thr
1               5                  10                  15

Leu Cys Leu Ala Thr Cys Gln Ser Leu Gln Glu Glu Thr Val Arg Lys
            20                  25                  30

Gly Pro Ala Gly Asp Arg Gly Pro Arg Gly Glu Arg Gly Pro Pro Gly
        35                  40                  45

Pro Pro Gly Arg Asp Gly Glu Asp Gly Pro Thr Gly Pro Pro Gly Pro
    50                  55                  60

Pro Gly Pro Pro Gly Pro Pro Gly Leu Gly Gly Asn Phe Ala Ala Gln
65                  70                  75                  80

Tyr Asp Gly Lys Gly Val Gly Leu Gly Pro Gly Pro Met Gly Leu Met
                85                  90                  95

Gly Pro Arg Gly Pro Pro Gly Ala Ala Gly Ala Pro Gly Pro Gln Gly
            100                 105                 110

Phe Gln Gly Pro Ala Gly Glu Pro Gly Glu Pro Gly Gln Thr Gly Pro
        115                 120                 125

Ala Gly Ala Pro Gly Pro His Gly Pro Val Gly Pro Ala Gly Lys His
    130                 135                 140
```

Gly Asn Arg Gly Glu Thr Gly Pro Ser Gly Pro Val Gly Pro Ala Gly
145 150 155 160

Ala Val Gly Pro Arg Gly Pro Ser Gly Pro Gln Gly Ile Arg Gly Asp
165 170 175

Lys Gly Glu Pro Gly Glu Lys Gly Pro Arg Gly Leu Pro Gly Phe Lys
180 185 190

Gly His Asn Gly Leu Gln Gly Leu Pro Gly Ile Ala Gly His His Gly
195 200 205

Asp Gln Gly Ala Pro Gly Ser Val Gly Pro Ala Gly Pro Arg Gly Pro
210 215 220

Ala Gly Pro Ser Gly Pro Ala Gly Lys Asp Gly Arg Thr Gly His Pro
225 230 235 240

Gly Thr Val Gly Pro Ala Gly Ile Arg Gly Pro Gln Gly His Gln Gly
245 250 255

Pro Ala Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Val
260 265 270

Ser Gly Gly Gly Tyr Asp Phe Gly Tyr Asp Gly Asp Phe Tyr Arg Ala
275 280 285

Asp Gln Pro Arg Ser Ala Pro Ser Leu Arg Pro Lys Asp Tyr Glu Val
290 295 300

Asp Ala Thr Leu Lys Ser Leu Asn Asn Gln Ile Glu Thr Leu Leu Thr
305 310 315 320

Pro Glu Gly Ser Arg Lys Asn Pro Ala Arg Thr Cys Arg Asp Leu Arg
325 330 335

Leu Ser His Pro Glu Trp Ser Ser Gly Tyr Tyr Trp Ile Asp Pro Asn
340 345 350

Gln Gly Cys Thr Met Glu Ala Ile Lys Val Tyr Cys Asp Phe Pro Thr
355 360 365

Gly Glu Thr Cys Ile Arg Ala Gln Pro Glu Asn Ile Pro Ala Lys Asn
370 375 380

Trp Tyr Arg Ser Ser Lys Asp Lys Lys His Val Trp Leu Gly Glu Thr
385 390 395 400

Ile Asn Ala Gly Ser Gln Phe Glu Tyr Asn Val Glu Gly Val Thr Ser
405 410 415

Lys Glu Met Ala Thr Gln Leu Ala Phe Met Arg Leu Leu Ala Asn Tyr
420 425 430

Ala Ser Gln Asn Ile Thr Tyr His Cys Lys Asn Ser Ile Ala Tyr Met
435 440 445

Asp Glu Glu Thr Gly Asn Leu Lys Lys Ala Val Ile Leu Gln Gly Ser
450 455 460

Asn Asp Val Glu Leu Val Ala Glu Gly Asn Ser Arg Phe Thr Tyr Thr
465 470 475 480

Val Leu Val Asp Gly Cys Ser Lys Lys Thr Asn Glu Trp Gly Lys Thr
485 490 495

Ile Ile Glu Tyr Lys Thr Asn Lys Pro Ser Arg Leu Pro Phe Leu Asp
500 505 510

Ile Ala Pro Leu Asp Ile Gly Gly Ala Asp His Glu Phe Phe Val Asp
515 520 525

Ile Gly Pro Val Cys Phe Lys
530 535

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 626 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Met Ser Phe Val Gln Lys Gly Ser Trp Leu Leu Leu Ala Leu Leu
1 5 10 15

His Pro Thr Ile Ile Leu Ala Gln Gln Glu Ala Val Glu Gly Gly Cys
20 25 30

Ser His Leu Gly Gln Ser Tyr Ala Asp Arg Asp Val Trp Lys Pro Glu
35 40 45

Pro Cys Gln Ile Cys Val Cys Asp Ser Gly Ser Val Leu Cys Asp Asp
50 55 60

Ile Ile Cys Asp Asp Gln Glu Leu Asp Cys Pro Asn Pro Glu Ile Pro
65 70 75 80

Phe Gly Glu Cys Cys Ala Val Cys Pro Gln Pro Pro Thr Ala Pro Thr
85 90 95

Arg Pro Pro Asn Gly Gln Gly Pro Gln Gly Pro Lys Gly Asp Pro Gly
100 105 110

Pro Pro Gly Ile Pro Gly Arg Asn Gly Asp Pro Gly Ile Pro Gly Gln

115 120 125

Pro Gly Ser Pro Gly Ser Pro Gly Pro Pro Gly Ile Cys Glu Ser Cys
130 135 140

Pro Thr Gly Pro Gln Asn Tyr Ser Pro Gln Tyr Asp Ser Tyr Asp Val
145 150 155 160

Lys Ser Gly Val Ala Val Gly Gly Leu Ala Gly Tyr Pro Gly Pro Ala
165 170 175

Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Thr Ser Gly His Pro Gly
180 185 190

Ser Pro Gly Ser Pro Gly Tyr Gln Gly Pro Pro Gly Glu Pro Gly Gln
195 200 205

Ala Gly Pro Ser Gly Pro Pro Gly Pro Pro Gly Ala Ile Gly Pro Ser
210 215 220

Gly Pro Ala Gly Lys Asp Gly Glu Ser Gly Arg Pro Gly Arg Pro Gly
225 230 235 240

Glu Arg Gly Leu Pro Gly Pro Pro Gly Ile Lys Gly Pro Ala Gly Ile
245 250 255

Pro Gly Phe Pro Gly Met Lys Gly His Arg Gly Phe Asp Gly Arg Asn
260 265 270

Gly Glu Lys Gly Glu Thr Gly Ala Pro Gly Leu Lys Gly Glu Asn Gly
275 280 285

Leu Pro Gly Glu Asn Gly Ala Pro Gly Pro Met Gly Pro Arg Gly Ala
290 295 300

Pro Gly Glu Arg Gly Arg Pro Gly Leu Pro Gly Ala Ala Gly Ala Arg

305 310 315 320

Gly Asn Asp Gly Ala Arg Gly Asn Arg Gly Glu Arg Gly Ser Glu Gly
325 330 335

Ser Pro Gly His Pro Gly Gln Pro Gly Pro Pro Gly Pro Pro Gly Ala
340 345 350

Pro Gly Pro Cys Cys Gly Gly Val Gly Ala Ala Ala Ile Ala Gly Ile
355 360 365

Gly Gly Glu Lys Ala Gly Gly Phe Ala Pro Tyr Tyr Gly Asp Glu Pro
370 375 380

Met Asp Phe Lys Ile Asn Thr Asp Glu Ile Met Thr Ser Leu Lys Ser
385 390 395 400

Val Asn Gly Gln Ile Glu Ser Leu Ile Ser Pro Asp Gly Ser Arg Lys
405 410 415

Asn Pro Ala Arg Asn Cys Arg Asp Leu Lys Phe Cys His Pro Glu Leu
420 425 430

Lys Ser Gly Glu Tyr Trp Val Asp Pro Asn Gln Gly Cys Lys Leu Asp
435 440 445

Ala Ile Lys Val Phe Cys Asn Met Glu Thr Gly Glu Thr Cys Ile Ser
450 455 460

Ala Asn Pro Leu Asn Val Pro Arg Lys His Trp Trp Thr Asp Ser Ser
465 470 475 480

Ala Glu Lys Lys His Val Trp Phe Gly Glu Ser Met Asp Gly Gly Phe
485 490 495

Gln Phe Ser Tyr Gly Asn Pro Glu Leu Pro Glu Asp Val Leu Asp Val

500 505 510

Gln Leu Ala Phe Leu Arg Leu Leu Ser Ser Arg Ala Ser Gln Asn Ile
515 520 525

Thr Tyr His Cys Lys Asn Ser Ile Ala Tyr Met Asp Gln Ala Ser Gly
530 535 540

Asn Val Lys Lys Ala Leu Lys Leu Met Gly Ser Asn Glu Gly Glu Phe
545 550 555 560

Lys Ala Glu Gly Asn Ser Lys Phe Thr Tyr Thr Val Leu Glu Asp Gly
565 570 575

Cys Thr Lys His Thr Gly Glu Trp Ser Lys Thr Val Phe Glu Tyr Arg
580 585 590

Thr Arg Lys Ala Val Arg Leu Pro Ile Val Asp Ile Ala Pro Tyr Asp
595 600 605

Ile Gly Gly Pro Asp Gln Glu Phe Gly Val Asp Val Gly Pro Val Cys
610 615 620

Phe Leu
625

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 623 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
Met Met Ser Phe Val Gln Lys Gly Ser Trp Leu Leu Leu Ala Leu Leu
1               5                  10                  15

His Pro Thr Ile Ile Leu Ala Gln Gln Glu Ala Val Glu Gly Gly Cys
            20                  25                  30

Ser His Leu Gly Gln Ser Tyr Ala Asp Arg Asp Val Trp Lys Pro Glu
        35                  40                  45

Pro Cys Gln Ile Cys Val Cys Asp Ser Gly Ser Val Leu Cys Asp Asp
    50                  55                  60

Ile Ile Cys Asp Asp Gln Glu Leu Asp Cys Pro Asn Pro Glu Ile Pro
65                  70                  75                  80

Phe Gly Glu Cys Cys Ala Val Cys Pro Gln Pro Pro Thr Ala Pro Thr
                85                  90                  95

Arg Pro Pro Asn Gly Gln Gly Pro Gln Gly Pro Lys Gly Asp Pro Gly
            100                 105                 110

Pro Pro Gly Ile Pro Gly Arg Asn Gly Asp Pro Gly Ile Pro Gly Gln
        115                 120                 125

Pro Gly Ser Pro Gly Ser Pro Gly Pro Pro Gly Ile Cys Glu Ser Cys
    130                 135                 140

Pro Thr Gly Pro Gln Asn Tyr Ser Pro Gln Tyr Asp Ser Tyr Asp Val
145                 150                 155                 160

Lys Ser Gly Val Ala Val Gly Gly Leu Ala Gly Tyr Pro Gly Pro Ala
                165                 170                 175

Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Thr Ser Gly His Pro Gly
            180                 185                 190
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Ser Pro Gly Ser Pro Gly Tyr Gln Gly Pro Pro Gly Glu Pro Gly Gln
195 200 205

Ala Gly Pro Ser Gly Pro Pro Gly Pro Pro Gly Ala Ile Gly Pro Ser
210 215 220

Gly Pro Ala Gly Lys Asp Gly Glu Ser Gly Arg Pro Gly Arg Pro Gly
225 230 235 240

Glu Arg Gly Leu Pro Gly Pro Pro Gly Ile Lys Gly Pro Ala Gly Ile
245 250 255

Pro Gly Phe Pro Gly Met Lys Gly His Arg Gly Phe Asp Gly Arg Asn
260 265 270

Gly Glu Lys Gly Glu Thr Gly Ala Pro Gly Leu Lys Gly Glu Asn Gly
275 280 285

Leu Pro Gly Glu Asn Gly Ala Pro Gly Pro Met Gly Pro Arg Gly Ala
290 295 300

Pro Gly Glu Arg Gly Arg Pro Gly Leu Pro Gly Ala Ala Gly Ala Arg
305 310 315 320

Gly Asn Asp Gly Ala Arg Gly Asn Arg Gly Glu Arg Gly Ser Glu Gly
325 330 335

Ser Pro Gly His Pro Gly Gln Pro Gly Pro Pro Gly Pro Pro Gly Ala
340 345 350

Pro Gly Pro Cys Cys Gly Gly Val Gly Ala Ala Ala Ile Ala Gly Ile
355 360 365

Gly Gly Glu Lys Ala Gly Gly Phe Ala Asp Gln Arg Ser Ala Pro Ser
370 375 380

Leu Arg Pro Lys Asp Tyr Glu Val Asp Ala Thr Leu Lys Ser Leu Asn
385 390 395 400

Asn Gln Ile Glu Thr Leu Leu Thr Pro Glu Gly Ser Arg Lys Asn Pro
405 410 415

Ala Arg Thr Cys Arg Asp Leu Arg Leu Ser His Pro Glu Trp Ser Ser
420 425 430

Gly Tyr Tyr Trp Ile Asp Pro Asn Gln Gly Cys Thr Met Glu Ala Ile
435 440 445

Lys Val Tyr Cys Asp Phe Pro Thr Gly Glu Thr Cys Ile Arg Ala Gln
450 455 460

Pro Glu Asn Ile Pro Ala Lys Asn Trp Tyr Arg Ser Ser Lys Asp Lys
465 470 475 480

Lys His Val Trp Leu Gly Glu Thr Ile Asn Ala Gly Ser Gln Phe Glu
485 490 495

Tyr Asn Val Glu Gly Val Thr Ser Lys Glu Met Ala Thr Gln Leu Ala
500 505 510

Phe Met Arg Leu Leu Ala Asn Tyr Ala Ser Gln Asn Ile Thr Tyr His
515 520 525

Cys Lys Asn Ser Ile Ala Tyr Met Asp Glu Glu Thr Gly Asn Leu Lys
530 535 540

Lys Ala Val Ile Leu Gln Gly Ser Asn Asp Val Glu Leu Val Ala Glu
545 550 555 560

Gly Asn Ser Arg Phe Thr Tyr Thr Val Leu Val Asp Gly Cys Ser Lys
565 570 575

Lys Thr Asn Glu Trp Gly Lys Thr Ile Ile Glu Tyr Lys Thr Asn Lys
580 585 590

Pro Ser Arg Leu Pro Phe Leu Asp Ile Ala Pro Leu Asp Ile Gly Gly
595 600 605

Ala Asp His Glu Phe Phe Val Asp Ile Gly Pro Val Cys Phe Lys
610 615 620

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 537 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Leu Ser Phe Val Asp Thr Arg Thr Leu Leu Leu Leu Ala Val Thr
1 5 10 15

Leu Cys Leu Ala Thr Cys Gln Ser Leu Gln Glu Glu Thr Val Arg Lys
20 25 30

Gly Pro Ala Gly Asp Arg Gly Pro Arg Gly Glu Arg Gly Pro Pro Gly
35 40 45

Pro Pro Gly Arg Asp Gly Glu Asp Gly Pro Thr Gly Pro Pro Gly Pro
50 55 60

Pro Gly Pro Pro Gly Pro Pro Gly Leu Gly Gly Asn Phe Ala Ala Gln
65 70 75 80

Tyr Asp Gly Lys Gly Val Gly Leu Gly Pro Gly Pro Met Gly Leu Met

85 90 95

Gly Pro Arg Gly Pro Pro Gly Ala Ala Gly Ala Pro Gly Pro Gln Gly
100 105 110

Phe Gln Gly Pro Ala Gly Glu Pro Gly Glu Pro Gly Gln Thr Gly Pro
115 120 125

Ala Gly Ala Pro Gly Pro His Gly Pro Val Gly Pro Ala Gly Lys His
130 135 140

Gly Asn Arg Gly Glu Thr Gly Pro Ser Gly Pro Val Gly Pro Ala Gly
145 150 155 160

Ala Val Gly Pro Arg Gly Pro Ser Gly Pro Gln Gly Ile Arg Gly Asp
165 170 175

Lys Gly Glu Pro Gly Glu Lys Gly Pro Arg Gly Leu Pro Gly Phe Lys
180 185 190

Gly His Asn Gly Leu Gln Gly Leu Pro Gly Ile Ala Gly His His Gly
195 200 205

Asp Gln Gly Ala Pro Gly Ser Val Gly Pro Ala Gly Pro Arg Gly Pro
210 215 220

Ala Gly Pro Ser Gly Pro Ala Gly Lys Asp Gly Arg Thr Gly His Pro
225 230 235 240

Gly Thr Val Gly Pro Ala Gly Ile Arg Gly Pro Gln Gly His Gln Gly
245 250 255

Pro Ala Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Val
260 265 270

Ser Gly Gly Gly Tyr Asp Phe Gly Tyr Asp Gly Asp Phe Tyr Arg Ala

275 280 285

Pro Tyr Tyr Gly Asp Glu Pro Met Asp Phe Lys Ile Asn Thr Asp Glu
290 295 300

Ile Met Thr Ser Leu Lys Ser Val Asn Gly Gln Ile Glu Ser Leu Ile
305 310 315 320

Ser Pro Asp Gly Ser Arg Lys Asn Pro Ala Arg Asn Cys Arg Asp Leu
325 330 335

Lys Phe Cys His Pro Glu Leu Lys Ser Gly Glu Tyr Trp Val Asp Pro
340 345 350

Asn Gln Gly Cys Lys Leu Asp Ala Ile Lys Val Phe Cys Asn Met Glu
355 360 365

Thr Gly Glu Thr Cys Ile Ser Ala Asn Pro Leu Asn Val Pro Arg Lys
370 375 380

His Trp Trp Thr Asp Ser Ser Ala Glu Lys Lys His Val Trp Phe Gly
385 390 395 400

Glu Ser Met Asp Gly Gly Phe Gln Phe Ser Tyr Gly Asn Pro Glu Leu
405 410 415

Pro Glu Asp Val Leu Asp Val Gln Leu Ala Phe Leu Arg Leu Leu Ser
420 425 430

Ser Arg Ala Ser Gln Asn Ile Thr Tyr His Cys Lys Asn Ser Ile Ala
435 440 445

Tyr Met Asp Gln Ala Ser Gly Asn Val Lys Lys Ala Leu Lys Leu Met
450 455 460

Gly Ser Asn Glu Gly Glu Phe Lys Ala Glu Gly Asn Ser Lys Phe Thr

465 470 475 480

Tyr Thr Val Leu Glu Asp Gly Cys Thr Lys His Thr Gly Glu Trp Ser
485 490 495

Lys Thr Val Phe Glu Tyr Arg Thr Arg Lys Ala Val Arg Leu Pro Ile
500 505 510

Val Asp Ile Ala Pro Tyr Asp Ile Gly Gly Pro Asp Gln Glu Phe Gly
515 520 525

Val Asp Val Gly Pro Val Cys Phe Leu
530 535

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 534 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met Leu Ser Phe Val Asp Thr Arg Thr Leu Leu Leu Leu Ala Val Thr
1 5 10 15

Leu Cys Leu Ala Thr Cys Gln Ser Leu Gln Glu Glu Thr Val Arg Lys
20 25 30

Gly Pro Ala Gly Asp Arg Gly Pro Arg Gly Glu Arg Gly Pro Pro Gly
35 40 45

Pro Pro Gly Arg Asp Gly Glu Asp Gly Pro Thr Gly Pro Pro Gly Pro
50 55 60

Pro Gly Pro Pro Gly Pro Pro Gly Leu Gly Gly Asn Phe Ala Ala Gln
65 70 75 80

Tyr Asp Gly Lys Gly Val Gly Leu Gly Pro Gly Pro Met Gly Leu Met
85 90 95

Gly Pro Arg Gly Pro Pro Gly Ala Ala Gly Ala Pro Gly Pro Gln Gly
100 105 110

Phe Gln Gly Pro Ala Gly Glu Pro Gly Glu Pro Gly Gln Thr Gly Pro
115 120 125

Gly Ala Pro Gly Pro His Gly Pro Val Gly Pro Ala Gly Lys His Gly
130 135 140

Asn Arg Gly Glu Thr Gly Pro Ser Gly Pro Val Gly Pro Ala Gly Ala
145 150 155 160

Val Gly Pro Arg Gly Pro Ser Gly Pro Gln Gly Ile Arg Gly Asp Lys
165 170 175

Gly Glu Pro Gly Glu Lys Gly Pro Arg Gly Leu Pro Gly Phe Lys Gly
180 185 190

His Asn Gly Leu Gln Gly Leu Pro Gly Ile Ala Gly His His Gly Asp
195 200 205

Gln Gly Ala Pro Gly Ser Val Gly Pro Ala Gly Pro Arg Gly Pro Ala
210 215 220

Gly Pro Ser Gly Pro Ala Gly Lys Asp Gly Arg Thr Gly His Pro Gly
225 230 235 240

Thr Val Gly Pro Ala Gly Ile Arg Gly Pro Gln Gly His Gln Gly Pro
245 250 255

Ala Gly Pro Pro Gly Pro Pro Gly Pro Leu Gly Pro Leu Gly Val Ser
260 265 270

Gly Gly Gly Tyr Asp Phe Gly Tyr Asp Gly Asp Phe Tyr Arg Ala Asp
275 280 285

Gln Pro Arg Ser Ala Pro Ser Leu Arg Pro Lys Asp Tyr Glu Val Asp
290 295 300

Ala Thr Leu Lys Ser Leu Asn Asn Gln Ile Glu Thr Leu Leu Thr Pro
305 310 315 320

Glu Gly Ser Arg Lys Asn Pro Ala Arg Thr Cys Arg Asp Leu Arg Leu
325 330 335

Ser His Pro Glu Trp Ser Ser Gly Tyr Tyr Trp Ile Asp Pro Asn Gln
340 345 350

Gly Cys Thr Met Glu Ala Ile Lys Val Tyr Cys Asp Phe Pro Thr Gly
355 360 365

Glu Thr Cys Ile Arg Ala Gln Pro Glu Asn Ile Pro Ala Lys Asn Trp
370 375 380

Tyr Arg Ser Ser Lys Asp Lys Lys His Val Trp Leu Gly Glu Thr Ile
385 390 395 400

Asn Ala Gly Ser Gln Phe Glu Tyr Gly Asn Pro Glu Leu Pro Glu Asp
405 410 415

Val Leu Asp Val Gln Leu Ala Phe Leu Arg Leu Leu Ser Ser Arg Ala
420 425 430

Ser Gln Asn Ile Thr Tyr His Cys Lys Asn Ser Ile Ala Tyr Met Asp
435 440 445

Glu Glu Thr Gly Asn Leu Lys Lys Ala Val Ile Leu Gln Gly Ser Asn
450 455 460

Asp Val Glu Leu Val Ala Glu Gly Asn Ser Arg Phe Thr Tyr Thr Val
465 470 475 480

Leu Val Asp Gly Cys Ser Lys Lys Thr Asn Glu Trp Gly Lys Thr Ile
485 490 495

Ile Glu Tyr Lys Thr Asn Lys Pro Ser Arg Leu Pro Phe Leu Asp Ile
500 505 510

Ala Pro Leu Asp Ile Gly Gly Ala Asp His Glu Phe Phe Val Asp Ile
515 520 525

Gly Pro Val Cys Phe Lys
530

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Gly Asn Pro Glu Leu Pro Glu Asp Val Leu Asp Val Gln Leu Ala Phe
1 5 10 15

Leu Arg Leu Leu Ser Ser Arg
20

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Gly Gly Gln Gly Ser Asp Pro Ala Asp Val Ala Ile Gln Leu Thr Phe
1 5 10 15

Leu Arg Leu Met Ser Thr Glu
20

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Asn Val Glu Gly Val Thr Ser Lys Glu Met Ala Thr Gln Leu Ala Phe
1 5 10 15

Met Arg Leu Leu Ala Asn Tyr
20

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Gly Asp Asp Asn Leu Ala Pro Asn Thr Ala Asn Val Gln Met Thr Phe
1 5 10 15

Leu Arg Leu Leu Ser Thr Glu
20

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Val Asp Ala Glu Gly Asn Pro Val Gly Val Val Gln Met Thr Phe Leu
1 5 10 15

Arg Leu Leu Ser Ala Ser
20

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Gly Asp His Gln Ser Pro Asn Thr Ala Ile Thr Gln Met Thr Phe Leu
1 5 10 15

Arg Leu Leu Ser Lys Glu
20

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Leu Asp Val Glu Gly Asn Ser Ile Asn Met Val Gln Met Thr Phe Leu
1 5 10 15

Lys Leu Leu Thr Ala Ser
20

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Val Asp Ser Glu Gly Ser Pro Val Gly Val Val Gln Leu Thr Phe Leu
1 5 10 15

Arg Leu Leu Ser Val Ser
20

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 250 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Tyr Tyr Arg Ala Asp Asp Ala Asn Val Val Arg Asp Arg Asp Leu Glu
1 5 10 15

Val Asp Thr Thr Leu Lys Ser Leu Ser Gln Gln Ile Glu Asn Ile Arg
20 25 30

Ser Pro Glu Gly Ser Arg Lys Asn Pro Ala Arg Thr Cys Arg Asp Leu
35 40 45

Lys Met Cys His Ser Asp Trp Lys Ser Gly Glu Tyr Trp Ile Asp Pro
50 55 60

Asn Gln Gly Cys Asn Leu Asp Ala Ile Lys Val Phe Cys Asn Met Glu
65 70 75 80

Thr Gly Glu Thr Cys Val Tyr Pro Thr Gln Pro Ser Val Ala Gln Lys
85 90 95

Asn Trp Tyr Ile Ser Lys Asn Pro Lys Asp Lys Arg His Val Trp Phe
100 105 110

Gly Glu Ser Met Thr Asp Gly Phe Gln Phe Glu Tyr Gly Gly Gln Gly
115 120 125

Ser Asp Pro Ala Asp Val Ala Ile Gln Leu Thr Phe Leu Arg Leu Met
130 135 140

Ser Thr Glu Ala Ser Gln Asn Ile Thr Tyr His Cys Lys Asn Ser Val
145 150 155 160

Ala Tyr Met Asp Gln Gln Thr Gly Asn Leu Lys Lys Ala Leu Leu Leu
165 170 175

Lys Gly Ser Asn Glu Ile Glu Ile Arg Ala Glu Gly Asn Ser Arg Phe
180 185 190

Thr Tyr Ser Val Thr Val Asp Gly Cys Thr Ser His Thr Gly Ala Trp
195 200 205

Gly Lys Thr Val Ile Glu Tyr Lys Thr Thr Lys Thr Ser Arg Leu Pro
210 215 220

Ile Ile Asp Val Ala Pro Leu Asp Val Gly Ala Pro Asp Gln Glu Phe
225 230 235 240

Gly Phe Asp Val Gly Pro Val Cys Phe Leu
245 250

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "PCR primer sequence"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

AATGGAGCTC CTGGACCCAT G 21

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "PCR primer sequence"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

AGGTGCTGAG CGAGGCTGGT CGGCAAAACC GCCAGCTTTT TC 42

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "PCR primer sequence"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

CTGCTAGGTA CCAAATGGAA GGATTCAGCT TT 32

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "PCR primer sequence"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GACCAGCCTC GCTCAGCA 18

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1608 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

ATGCTCAGCT TTGTGGATAC GCGGACTTTG TTGCTGCTTG CAGTAACCTT ATGCCTAGCA 60

ACATGCCAAT CTTTACAAGA GGAAACTGTA AGAAAGGGCC CAGCCGGAGA TAGAGGACCA 120

CGTGGAGAAA GGGGTCCACC AGGCCCCCCA GGCAGAGATG GTGAAGATGG TCCCACAGGC 180

CCTCCTGGTC CACCTGGTCC TCCTGGCCCC CCTGGTCTCG GTGGGAACTT TGCTGCTCAG 240

TATGATGGAA AAGGAGTTGG ACTTGGCCCT GGACCAATGG GCTTAATGGG ACCTAGAGGC 300

CCACCTGGTG CAGCTGGAGC CCCAGGCCCT CAAGGTTTCC AAGGACCTGC TGGTGAGCCT 360

GGTGAACCTG GTCAAACTGG TCCTGCAGGT GCACCTGGTC CTCATGGCCC CGTGGGTCCT 420

GCTGGCAAAC ATGGAAACCG TGGTGAAACT GGTCCTTCTG GTCCTGTTGG TCCTGCTGGT 480

GCTGTTGGCC CAAGAGGTCC TAGTGGCCCA CAAGGCATTC GTGGCGATAA GGGAGAGCCC 540

GGTGAAAAGG GGCCCAGAGG TCTTCCTGGC TTCAAGGGAC ACAATGGATT GCAAGGTCTG 600

CCTGGTATCG CTGGTCACCA TGGTGATCAA GGTGCTCCTG GCTCCGTGGG TCCTGCTGGT 660

CCTAGGGGCC CTGCTGGTCC TTCTGGCCCT GCTGGAAAAG ATGGTCGCAC TGGACATCCT 720

GGTACGGTTG GACCTGCTGG CATTCGAGGC CCTCAGGGTC ACCAAGGCCC TGCTGGCCCC 780

CCTGGTCCCC CTGGCCCTCC TGGACCTCCA GGTGTAAGCG GTGGTGGTTA TGACTTTGGT 840

TACGATGGAG ACTTCTACAG GGCTGACCAG CCTCGCTCAG CACCTTCTCT CAGACCCAAG 900

GACTATGAAG TTGATGCTAC TCTGAAGTCT CTCAACAACC AGATTGAGAC CCTTCTTACT 960

CCTGAAGGCT CTAGAAAGAA CCCAGCTCGC ACATGCCGTG ACTTGAGACT CAGCCACCCA 1020

GAGTGGAGCA GCGGTTACTA CTGGATTGAC CCCAACCAAG GATGCACTAT GGAAGCCATC 1080

AAAGTATACT GTGATTTCCC TACCGGCGAA ACCTGTATCC GGGCCCAACC TGAAAACATC 1140

CCAGCCAAGA ACTGGTATAG GAGCTCCAAG GACAAGAAAC ACGTCTGGCT AGGAGAAACT 1200

ATCAATGCTG GCAGCCAGTT TGAATATAAT GTTGAAGGAG TGACTTCCAA GGAAATGGCT 1260

ACCCAACTTG CCTTCATGCG CCTGCTGGCC AACTATGCCT CTCAGAACAT CACCTACCAC 1320

TGCAAGAACA GCATTGCATA CATGGATGAG GAGACTGGCA ACCTGAAAAA GGCTGTCATT 1380

CTACAGGGCT CTAATGATGT TGAACTTGTT GCTGAGGGCA ACAGCAGGTT CACTTACACT 1440

GTTCTTGTAG ATGGCTGCTC TAAAAAGACA AATGAATGGG GAAAGACAAT CATTGAATAC 1500

AAAACAAATA AGCCATCACG CCTGCCCTTC CTTGATATTG CACCTTTGGA CATCGGTGGT 1560

GCTGACCATG AATTCTTTGT GGACATTGGC CCAGTCTGTT TCAAATAA 1608

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1881 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

ATGATGAGCT TTGTGCAAAA GGGGAGCTGG CTACTTCTCG CTCTGCTTCA TCCCACTATT 60

ATTTTGGCAC AACAGGAAGC TGTTGAAGGA GGATGTTCCC ATCTTGGTCA GTCCTATGCG 120

GATAGAGATG TCTGGAAGCC AGAACCATGC CAAATATGTG TCTGTGACTC AGGATCCGTT 180

CTCTGCGATG ACATAATATG TGACGATCAA GAATTAGACT GCCCCAACCC AGAAATTCCA 240

TTTGGAGAAT GTTGTGCAGT TTGCCCACAG CCTCCAACTG CTCCTACTCG CCCTCCTAAT 300

GGTCAAGGAC CTCAAGGCCC CAAGGGAGAT CCAGGCCCTC CTGGTATTCC TGGGAGAAAT 360

GGTGACCCTG GTATTCCAGG ACAACCAGGG TCCCCTGGTT CTCCTGGCCC CCCTGGAATC 420

TGTGAATCAT GCCCTACTGG TCCTCAGAAC TATTCTCCCC AGTATGATTC ATATGATGTC 480

AAGTCTGGAG TAGCAGTAGG AGGACTCGCA GGCTATCCTG GACCAGCTGG CCCCCCAGGC 540

CCTCCCGGTC CCCCTGGTAC ATCTGGTCAT CCTGGTTCCC CTGGATCTCC AGGATACCAA 600

GGACCCCCTG GTGAACCTGG GCAAGCTGGT CCTTCAGGCC CTCCAGGACC TCCTGGTGCT 660

ATAGGTCCAT CTGGTCCTGC TGGAAAAGAT GGAGAATCAG GTAGACCCGG ACGACCTGGA 720

GAGCGAGGAT TGCCTGGACC TCCAGGTATC AAAGGTCCAG CTGGGATACC TGGATTCCCT 780

GGTATGAAAG GACACAGAGG CTTCGATGGA CGAAATGGAG AAAAGGGTGA AACAGGTGCT 840

CCTGGATTAA AGGGTGAAAA TGGTCTTCCA GGCGAAAATG GAGCTCCTGG ACCCATGGGT 900

CCAAGAGGGG CTCCTGGTGA GCGAGGACGG CCAGGACTTC CTGGGGCTGC AGGTGCTCGG 960

GGTAATGACG GTGCTCGAGG TAACAGAGGT GAAAGAGGAT CTGAGGGCTC CCCAGGCCAC 1020

CCAGGGCAAC CAGGCCCTCC TGGACCTCCT GGTGCCCCTG GTCCTTGCTG TGGTGGTGTT 1080

GGAGCCGCTG CCATTGCTGG GATTGGAGGT GAAAAAGCTG GCGGTTTTGC CCCGTATTAT 1140

GGAGATGAAC CAATGGATTT CAAAATCAAC ACCGATGAGA TTATGACTTC ACTCAAGTCT 1200

GTTAATGGAC AAATAGAAAG CCTCATTAGT CCTGATGGTT CTCGTAAAAA CCCCGCTAGA 1260

AACTGCAGAG ACCTGAAATT CTGCCATCCT GAACTCAAGA GTGGAGAATA CTGGGTTGAC 1320

CCTAACCAAG GATGCAAATT GGATGCTATC AAGGTATTCT GTAATATGGA AACTGGGGAA 1380

ACATGCATAA GTGCCAATCC TTTGAATGTT CCACGGAAAC ACTGGTGGAC AGATTCTAGT 1440

GCTGAGAAGA AACACGTTTG GTTTGGAGAG TCCATGGATG GTGGTTTTCA GTTTAGCTAC 1500

GGCAATCCTG AACTTCCTGA AGATGTCCTT GATGTGCAGC TGGCATTCCT TCGACTTCTC 1560

TCCAGCCGAG CTTCCCAGAA CATCACATAT CACTGCAAAA ATAGCATTGC ATACATGGAT 1620

CAGGCCAGTG GAAATGTAAA GAAGGCCCTG AAGCTGATGG GGTCAAATGA AGGTGAATTC 1680

AAGGCTGAAG GAAATAGCAA ATTCACCTAC ACAGTTCTGG AGGATGGTTG CACGAAACAC 1740

ACTGGGGAAT GGAGCAAAAC AGTCTTTGAA TATCGAACAC GCAAGGCTGT GAGACTACCT 1800

ATTGTAGATA TTGCACCCTA TGACATTGGT GGTCCTGATC AAGAATTTGG TGTGGACGTT 1860

GGCCCTGTTT GCTTTTTATA A 1881

CLAIMS

1. A molecule comprising at least a first moiety having the activity of a procollagen C-propeptide and a second moiety selected from any one of the group of an alien collagen α-chain and non-collagen materials, the first moiety being attached to the second moiety.

2. A molecule according to claim 1 wherein the first moiety comprises an existing C-propeptide or a molecule resulting from partial modification thereof or an analogue thereof.

3. A molecule according to claim 2 wherein the existing C-propeptide is selected from any one of the group of the proα1(I), proα2(I), proα1(II), proα1(III), proα1(V), proα2(V), proα3(V), proα1(XI), proα2(XI), and proα3(XI) proα chain C-propeptides.

4. A molecule according to claim 1 wherein the first moiety comprises a novel C-propeptide.

5. A molecule according to claim 4 wherein the C-propeptide comprises a C-propeptide substituted at the recognition site.

6. A molecule according to claim 5 wherein the C-propeptide has been substituted at the recognition site with the recognition sequence of an existing C-propeptide or a partially modified form thereof or an analogue thereof.

7. A molecule according to claim 6 wherein the C-propeptide has been substituted at the recognition site with the recognition sequence of the C-propeptide of any

one of the group of proα1(III), proα1(I), proα2(I), proα1(II), proα1(V), proα2(V), proα1(XI) and proα2(XI) proα chains.

8. A molecule according to claim 7 wherein the C-propeptide has been substituted at the recognition site with a recognition sequence having the sequence of any one of the group of SEQ ID NOs: 6-13.

9. A molecule according to claim 5 wherein the C-propeptide is substituted at the recognition site with a novel recognition sequence.

10. A molecule according to any one of claims 2-9 wherein the C-propeptide and/or the recognition sequence is that of a fibrillar proα chain.

11. A molecule according to any one of the preceding claims wherein the second moiety comprises at least a collagen α-chain.

12. A molecule according to claim 11 wherein the collagen α-chain is selected from any one of the group of proα1(I) chain, proα2(I) chain, proα1(II) chain, proα1(III) chain, proα1(V) chain, proα2(V) chain, proα3(V) chain, proα1(XI) chain, proα2(XI) chain, and proα3(XI) chain collagen α-chains.

13. A molecule according to any one of the preceding claims wherein the second moiety also comprises a proα chain N-propeptide.

14. A molecule according to claim 13 wherein the N-propeptide is selected from any one of the group of the proα1(I), proα2(I), proα1(II), proα1(III), proα1(V), proα2(V), proα3(V), proα1(XI), proα2(XI), and proα3(XI) proα chain N-propeptides.

15. A molecule according to claim 14 wherein it comprises a first moiety having the activity of the proα1(III) C-propeptide attached to a second moiety comprising the collagen α-chain and N-propeptide of the proα2(I) chain.

16. A molecule according to claim 13 wherein it has the sequence of SEQ ID NO: 4.

17. A molecule according to any one of the preceding claims wherein the first and second moieties are separated by intervening amino acid residues.

18. A collagen molecule comprising a non-natural combination of collagen α-chains.

19. A collagen fibril comprising collagen molecules according to claim 18.

20. A collagen fibre comprising collagen fibrils according to claim 19.

21. A molecule according to any one of the preceding claims for use in a method of treatment or diagnosis of the human or animal body.

22. A molecule according to claim 21 for use in the treatment of procollagen suicide.

23. A molecule according to claim 21 for use as an adhesive or an implant.

24. A molecule according to claim 21 wherein it is for use in promoting the healing of wounds or fibrotic disorders with reduced scarring.

25. A molecule according to claim 21 wherein it is for use in promoting the healing of chronic wounds.

26. A molecule according to any one of claims 1 to 20 for use in photography, brewing, foodstuffs, textiles or adhesives.

27. A method of treatment or diagnosis of the human or animal body comprising the use of a molecule according to any one of claims 1 to 25.

28. DNA encoding a molecule according to any one of claims 1-20.

29. An expression host transformed or transfected with DNA according to claim 28 operably linked to regulatory sequences sufficient to direct expression.

30. A transgenic animal whose genome comprises DNA according to claim 29 operably linked to regulatory sequences sufficient to direct expression.

31. A transgenic animal according to claim 30, the animal being a non-human placental mammal and the regulatory sequences directing expression in the milk of an adult female.

32. A method of producing a non-natural collagen, the method comprising harvesting the collagen from an expression host according claim 29 or a transgenic animal according to claim 30 or 31 and optionally subsequently purifying the non-natural collagen.

Figure 1

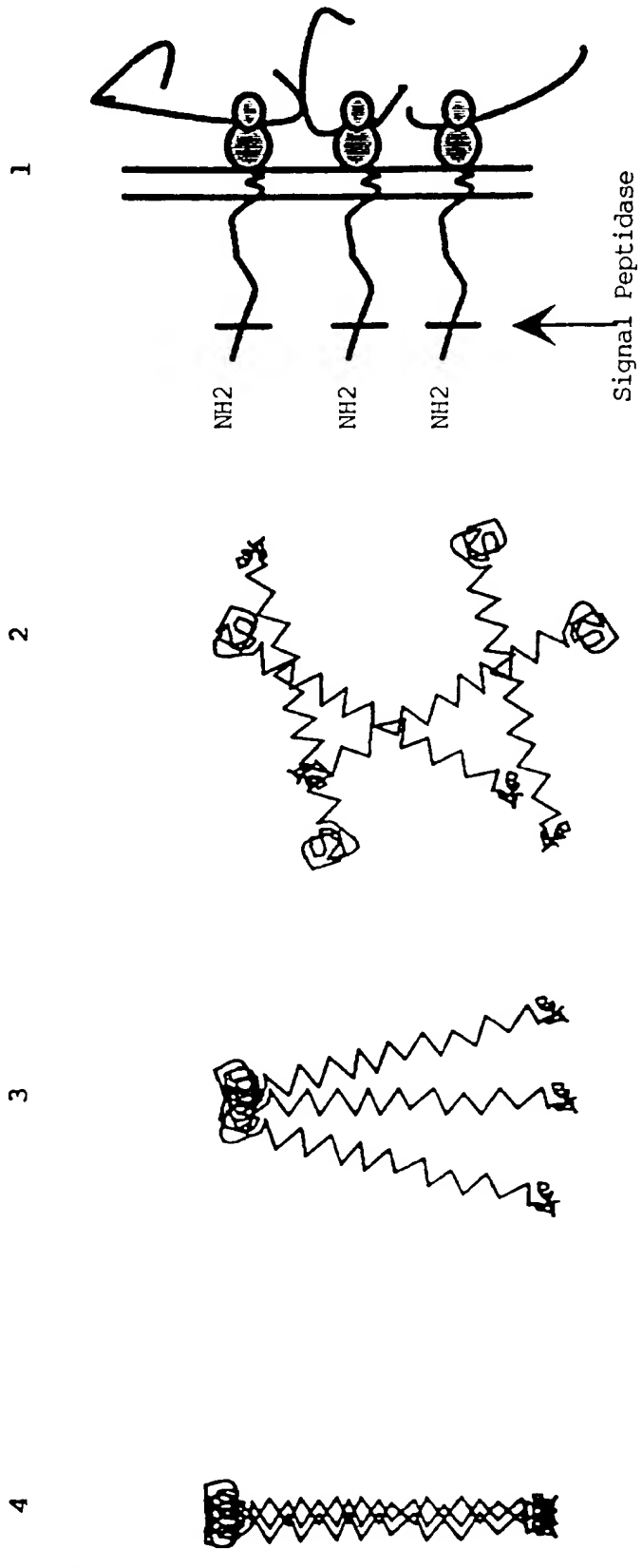


Figure 2

```
                  CP
                  CP
                 CP                A                                  1
alpha1(I)     YYRADD...A NVVRDRDLEV DTTLKSLSQQ IENIRSPEGS RKNPARTCRD
alpha2(I)     FYRADQPRSA PSLRPKDYEV DATLKSLNNQ IETLLTPEGS RKNPARTCRD
alpha1(III)   YYGDE....P MDFKINTDEI MTSLKSVNGQ IESLISPDGS RKNPARNCRD
                        ~    ~    #~  ~~###~~ # ## ~ ~#~## ###### ###

                    2                  3        4 F      5
alpha1(I)     LKMCHSDWKS GEYWIDPNQG CNLDAIKVFC NMETGETCVY PTQPSVAQKN
alpha2(I)     LRLSHPEWSS GYYWIDPNQG CTMEAIKVYC DFPTGETCIR AQPENIPAKN
alpha1(III)   LKFCHPELKS GEYWVDPNQG CKLDAIKVFC NMETGETCIS ANPLNVPRKH
              #~  #~~  # # ##~##### # ~~####~# ~  #####~  ~   ~~~ #~

                                          B           C
alpha1(I)     WYISKNPKDK RHVWFGESMT DGFQFEYGGQ GSDPADVAIQ LTFLRLMSTE
alpha2(I)     WYRS..SKDK KHVWLGETIN AGSQFEYNVE GVTSKEMATQ LAFMRLLANY
alpha1(III)   WW.TDSSAEK KHVWFGESMD GGFQFSYGNP ELPEDVLDVQ LAFLRLLSSR
              #~ ~  ~ ~# ~###~##~~   # ## #          ~  # #~#~##~~

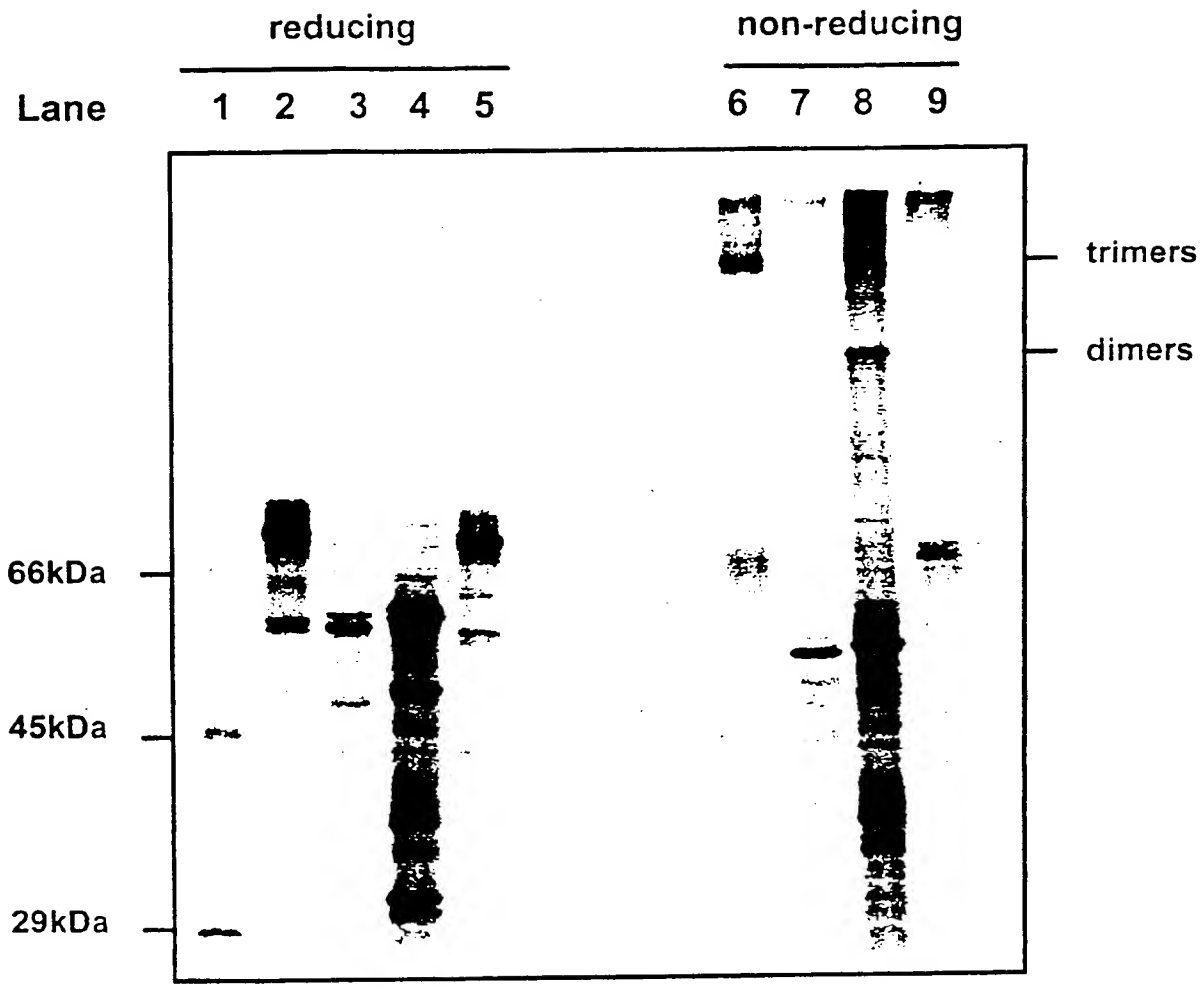
              G       6
alpha1(I)     ASQNITYHCK NSVAYMDQQT GNLKKALLLK GSNEIEIRAE GNSRFTYSVT
alpha2(I)     ASQNITYHCK NSIAYMDEET GNLKKAVILQ GSNDVELVAE GNSRFTYTVL
alpha1(III)   ASQNITYHCK NSIAYMDQAS GNVKKALKLM GSNEGEFKAE GNSKFTYTVL
              ########## ##~####~ ~ ##~###~ #  ###~ #~ ## ###~###~#

                7                                                   8
alpha1(I)     VDGCTSHTGA WGKTVIEYKT TKTSRLPIID VAPLDVGAPD QEFGFDVGPV CFL
alpha2(I)     VDGCSKKTNE WGKTIIEYKT NKPSRLPFLD IAPLDIGGAD HEFFVDIGPV CFK
alpha1(III)   EDGCTKHTGE WSKTVFEYRT RKAVRLPIVD IAPYDIGGPD QEFGVDVGPV CFL
               ###~  #   #~##~~##~#  #  ###~~# ~## #~#~~# ~##  #~### ##
```

Figure 3

```
alpha 1(I)     GGQGSDPADV AIQLTFLRLM STE
alpha 2(I)     NVEGVTSKEM ATQLAFMRLL ANY
alpha 1(II)    GDDNLAPNTA NVQMTFLRLL STE
alpha 1(III)   GNPELPEDVL DVQLAFLRLL SSR
alpha 1(V)     VDAEGNPVGV .VQMTFLRLL SAS
alpha 2(V)     GDHQSPNTAI .TQMTFLRLL SKE
alpha 1(XI)    LDVEGNSINM .VQMTFLKLL TAS
alpha 2(XI)    VDSEGSPVGV .VQLTFLRLL SVS
```

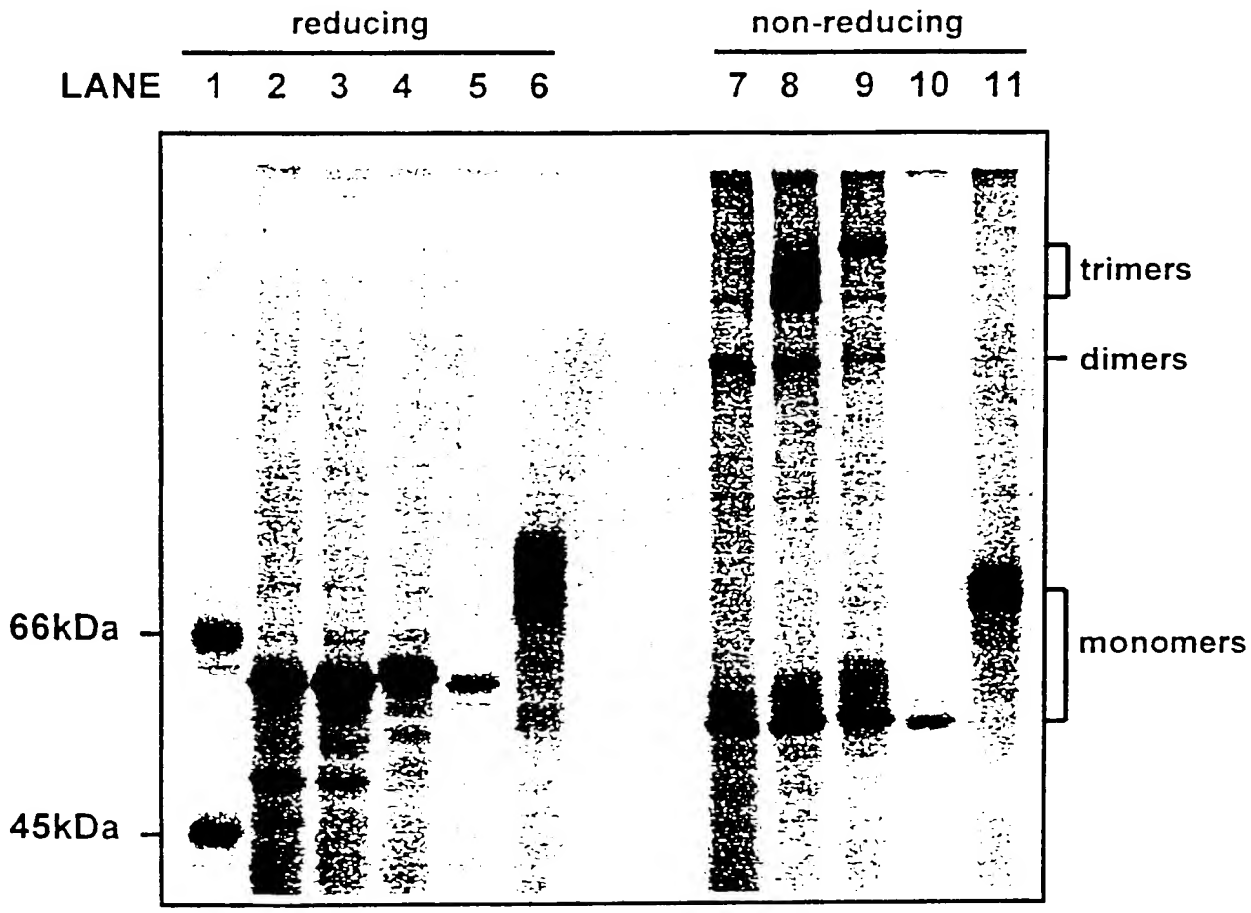
Figure 4



Lane	procollagen construct translated
2 and 6	α1(III)Δ1
3 and 7	α2(I)Δ1
4 and 8	α2(I):(III)CP
5 and 9	α1(III):(I)CP

Lane 1:- molecular weight markers

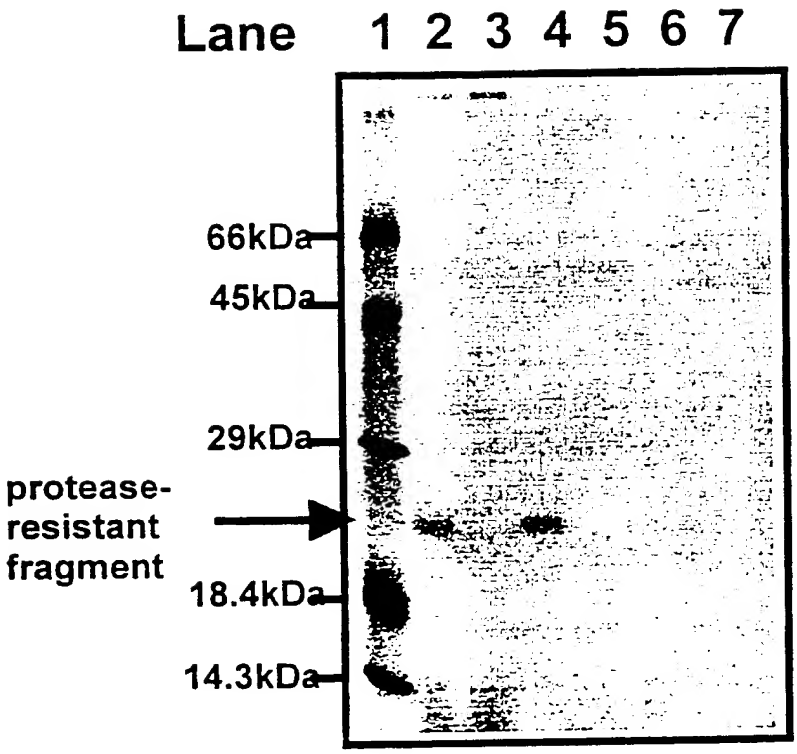
Figure 5



Lane	procollagen construct translated
2 and 7	A-join
3 and 8	F-join
4 and 9	B-join
5 and 10	C-join
6 and 11	recip-C-join

Lane 1:- molecular weight markers

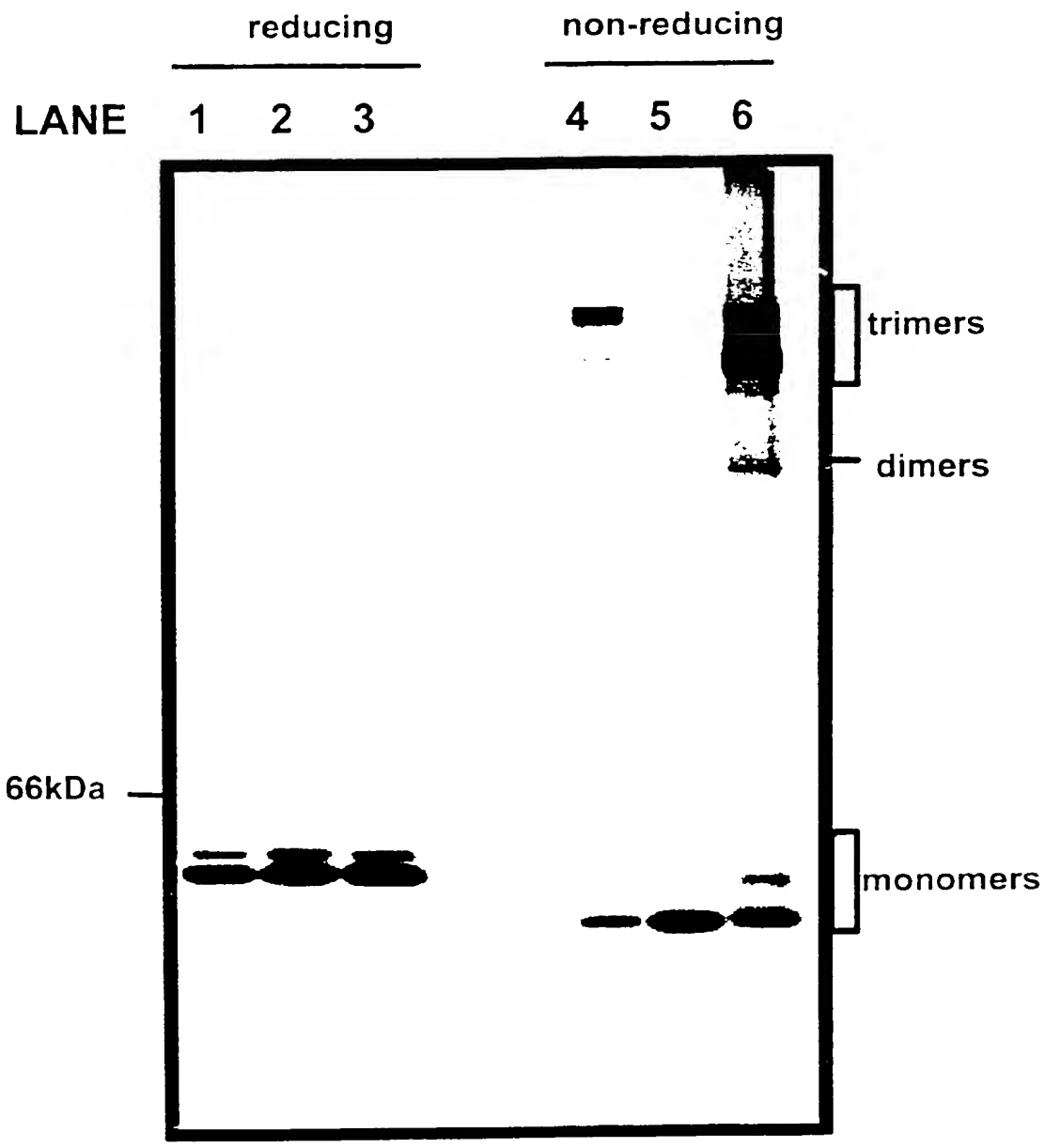
Figure 6



Lane	dipyridyl present	procollagen translated
2	No	proα2(I):(III)CP
3	Yes	proα2(I):(III)CP
4	No	BGR
5	Yes	BGR
6	No	proα1(III):(I)CP
7	Yes	proα1(III):(I)CP

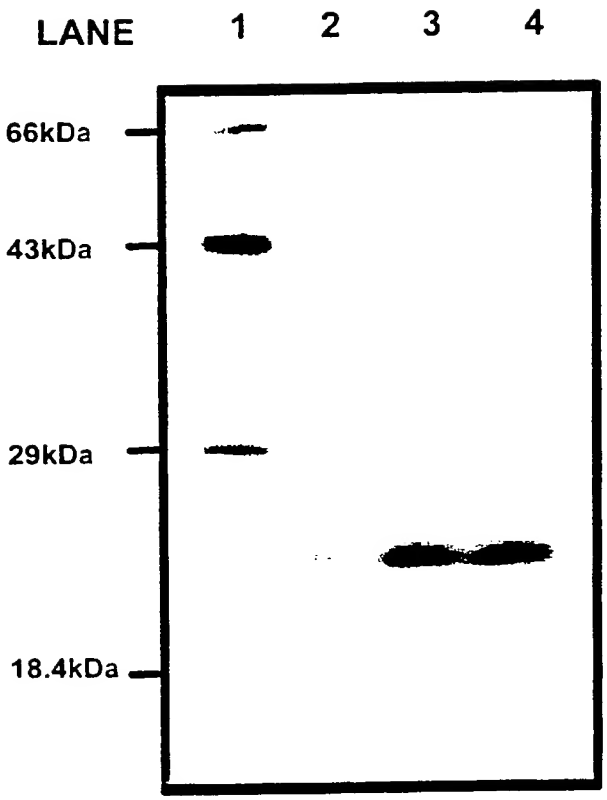
Lane 1:- molecular weight markers

Figure 7



Lanes	procollagen construct translated
1 and 4	BGR $^{s-c}$
2 and 5	BGR
3 and 6	BGR^{L-M}

Figure 8



lane	procollagen construct
2	BGR^{s-c}
3	BGR
4	BGR^{L-M}

Lane 1: molecular weight markers

INTERNATIONAL SEARCH REPORT

International Application No
PC./GB 96/02122

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12N15/62 C07K14/78 A01K67/027 A61K38/39
G01N33/68 A23L1/305 A61L27/00 D06M15/15 C12C5/02
G03C1/047

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,5 424 408 (REEDERS STEPHEN T ET AL) 13 June 1995 see claims ---	1,11, 18-21, 27-29,32
X	WO,A,93 07889 (UNIV JEFFERSON) 29 April 1993 cited in the application see claims --- -/--	18-21, 23-25, 28,29,32

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 January 1997	21. 01. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Delanghe, L

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INTERNATIONAL SEARCH REPORT

Inter- nonal Application No
PC1/GB 96/02122

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BIOCHEMISTRY, vol. 30, no. 29, 23 July 1991, EASTON, PA US, pages 7097-7104, XP000215689 KOU KATAYAMA ET AL.: "Regulation of extracellular matrix production by chemically synthesized subfragments of type I collagen carboxy propeptide." see the whole document	1
A	WO,A,94 16570 (COLLAGEN CORP) 4 August 1994 cited in the application see claims	1,30-32

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 96/02122

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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WO-A-9307889	29-04-93	AU-A-	3055592	21-05-93
		CA-A-	2121698	29-04-93
		EP-A-	0625048	23-11-94
		JP-T-	7501939	02-03-95
		US-A-	5405757	11-04-95
WO-A-9416570	04-08-94	AU-A-	6031794	15-08-94
		CA-A-	2152047	04-08-94
		EP-A-	0681431	15-11-95
		JP-T-	8506016	02-07-96